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Animal models to assess the abuse liability of tobacco products: Effects of smokeless tobacco extracts on intracranial self-stimulation

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ABSTRACT

Background: Preclinical models are needed to inform regulation of tobacco products by the Food and Drug Administration (FDA). Typically, animal models of tobacco addiction involve exposure to nicotine alone or nicotine combined with isolated tobacco constituents (e.g. minor alkaloids). The goal of this study was to develop a model using extracts derived from tobacco products that contain a range of tobacco constituents to more closely model product exposure in humans.
Methods: This study compared the addiction-related effects of nicotine alone and nicotine dose-equivalent concentrations of aqueous smokeless tobacco extracts on intracranial self-stimulation (ICSS) in rats. Extracts were prepared from Kodiak Wintergreen, a conventional product, or Camel Snus, a potential "modified risk tobacco product". Binding affinities of nicotine alone and extracts at various nicotinic acetylcholine receptor (nAChR) subtypes were also compared.
Results: Kodiak and Camel Snus extracts contained levels of minor alkaloids within the range of those shown to enhance nicotine's behavioral effects when studied in isolation. Nonetheless, acute injection of both extracts produced reinforcement-enhancing (ICSS threshold-decreasing) effects similar to those of nicotine alone at low to moderate nicotine doses, as well as similar reinforcement-attenuating/aversive (ICSS threshold-increasing) effects at high nicotine doses. Extracts and nicotine alone also had similar binding affinity at all nAChRs studied.
Conclusions: Relative nicotine content is the primary pharmacological determinant of the abuse liability of Kodiak and Camel Snus as measured using ICSS. These models may be useful to compare the relative abuse liability of other tobacco products and to model FDA-mandated changes in product performance standards.

1. Introduction

The 2009 Family Smoking Prevention and Tobacco Control Act provides the Food and Drug Administration (FDA) regulatory authority over tobacco products (U.S. Congress, 2009; Deyton et al., 2010; Hatsukami et al., 2012, 2010; Zeller and Hatsukami, 2009). Among many other provisions under this law, the FDA has the authority to set performance standards for current tobacco products, including reductions in nicotine yields or levels of other constituents, if deemed appropriate for protection of public health. Part of this process includes evaluating the relative abuse liability of new tobacco products prior to marketing to determine if they are substantially equivalent to current products. That is, it must be determined whether they have the same characteristics (e.g. ingredients, design) as currently marketed products; or have different characteristics, but do not pose a new or increased threat to public health (U.S. Congress, 2009). The tobacco industry has introduced several potential "modified risk tobacco products" (MRTPs) claimed to be safer than conventional tobacco products due to their lower

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levels of toxicants (e.g. tobacco-specific nitrosamines). However, they may not be safer in other respects, such as abuse liability (Hatsukami et al., 2012, 2007, 2010; Pederson and Nelson, 2007; Zeller and Hatsukami, 2009). Development of appropriate methodology for premarket evaluation of the relative abuse liability of potential MRTPs and other tobacco products is needed to inform FDA regulatory policy.

The Institute of Medicine has specifically recommended the use of animal models for the evaluation of tobacco products (Stratton et al., 2001), as they avoid limitations associated with human studies (e.g. inability to isolate the role of nicotine and other tobacco constituents from other factors). Animal models of tobacco addiction typically involve administration of nicotine alone or nicotine combined with other tobacco constituents (e.g. minor alkaloids, acetaldehyde) (Belluzzi et al., 2005; Clemens et al., 2009; Villegier et al., 2007). This approach is not sufficient to evaluate the abuse liability of tobacco products because as yet unidentified compounds may contribute (positively or negatively) to tobacco abuse. Moreover, it is the interaction of these compounds that ultimately determines the abuse liability of a product. Another limitation of many preclinical studies of isolated constituents is that the doses administered may not match the doses delivered during actual tobacco product use (Harris et al., 2012).

Animal models using extracts derived from tobacco or tobacco smoke and containing a comprehensive range of constituents would more accurately simulate tobacco product exposure in humans. Limited data address the feasibility and utility of this approach. Delivery of nicotine in extracts can enhance its addiction-related neurobiological and behavioral effects (Ambrose et al., 2007; Brennan et al., 2013a, 2014; Costello et al., 2014; Touiki et al., 2007), consistent with the ability of certain *isolated* non-nicotine constituents (e.g. minor alkaloids) to mimic or enhance nicotine's effects in these assays (e.g. Bardo et al., 1999; Belluzzi et al., 2005; Dwoskin et al., 1999; Foddai et al., 2004; Guillem et al., 2005; Villegier et al., 2007). Additional behavioral and neurobiological evaluation of tobacco extracts is needed to further develop this approach for the evaluation of tobacco products by the FDA.

Intracranial self-stimulation (ICSS) has been used extensively to study the effects of nicotine and other addictive drugs on brain reinforcement systems. At low to moderate doses, nicotine lowers the minimal (threshold) stimulation intensity that maintains ICSS, reflecting its ability to enhance the function of brain reinforcement pathways and, thereby, enhance the reinforcing effects of other stimuli (Caggiula et al., 2009; Chaudhri et al., 2006; Harrison et al., 2002; Huston-Lyons and Kornetsky, 1992; Kornetsky et al., 1979; Paterson et al., 2008; Wise, 2002). This is a particularly sensitive predictor of abuse liability, as false positives are extremely rare and some addictive drugs that do not have abuse liability in i.v. self-administration models (e.g. hallucinogens) still reduce ICSS thresholds (Wise, 1996, 2002; Wise et al., 1992). At high doses, nicotine attenuates the reinforcing effects of brain stimulation and increases ICSS thresholds, a putative marker of nicotine's acute aversive effects (Fowler et al., 2011; Kenny et al., 2003; Spiller et al., 2009). Nicotine's reinforcement-enhancing and aversive effects are both thought to influence likelihood or rate of tobacco use (Donny et al., 2003; Laviolette and van der Kooy, 2003; Liu et al., 2007; Sellings et al., 2008; Wilmouth and Spear, 2004). Supporting the sensitivity of ICSS, we found that delivery of a high dose of nicotine in a smokeless tobacco extract produced less aversive effects in this assay compared to nicotine alone (Harris et al., 2012).

The primary goal of this study was to compare the acute effects of nicotine alone and nicotine dose-equivalent concentrations of smokeless tobacco extracts on ICSS. In our previous study (Harris et al., 2012), extracts were prepared from Kodiak Wintergreen, a popular conventional product. An important limitation of that study was that animals were not experimentally naïve. It is also well established that constituent levels within the same tobacco product can vary substantially across time (Stepanov et al., 2014, 2012). Therefore, we first evaluated the acute effects of Kodiak extract on ICSS in an attempt to replicate our previous findings. In a separate experiment, extracts were prepared from Camel Snus, a widely marketed potential MRTP that has not been studied in a preclinical behavioral model. Levels of the behaviorally relevant minor alkaloids nornicotine, anabasine, and anatabine in Kodiak and Camel Snus extracts were also measured. Finally, binding affinities of extracts and nicotine alone at a panel of nicotinic acetylcholine receptor (nAChR) subtypes were compared. Affinities of formulations at α4ß2, α3ß4, and α7 nAChRs were of particular interest because of the important role of these nAChRs in tobacco addiction (Changeux, 2010; De Biasi and Salas, 2008; Fowler et al., 2008).

2. Methods

2.1. Animals

Experimentally naive male Holtzman Sprague Dawley rats (Harlan, Indianapolis, IN) weighing 250–300 g at arrival were housed individually in a temperature- and humidity-controlled colony room with unlimited access to water. Rats were housed under a reversed 12 h light/dark cycle and tested during the dark (active) phase. Beginning one week after arrival, rats were food-restricted to ≈18 g/day rat chow to facilitate operant performance and avoid detrimental effects of long-term ad libitum feeding on health. Protocols were approved by the Institutional Animal Care and Use Committee of the Minneapolis Medical Research Foundation in accordance with the 2011 NIH Guide for the Care and Use of Laboratory Animals and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (National Research Council 2003).

2.2. Drugs

Nicotine-alone solutions consisted of (-)-Nicotine bitartrate (Sigma Chemical Co., St. Louis, MO) dissolved in sterile saline. Aqueous tobacco extract was prepared from Kodiak Wintergreen or Camel Snus Winterchill smokeless tobacco products (purchased in the Minneapolis area between January, 2013, and January, 2014) using general procedures described elsewhere (Harris et al., 2012). Briefly, tobacco product was mixed with saline vehicle at a concentration of 400 mg/ml (Kodiak extract) or 200 mg/ml (Camel Snus extract) for 18 h using a tube tipper. The different concentrations of the extracts reflect the higher volume of saline required for preparation of extract from Camel Snus, which is considerably more absorbant than Kodiak. A saline extraction produces a similar alkaloid extraction profile as artificial saliva and simplifies extract preparation while avoiding toxicity (Harris et al., 2012). The resulting solution was filtered through gauze, centrifuged, and the supernate was filtered. The nicotine concentration was determined, and extract was diluted to the nicotine concentrations required for the current studies. The pH of all solutions was adjusted to 7.4 using dilute NaOH. Nicotine doses are expressed as the base. All injections were administered s.c. in a volume of 1 ml/kg.

2.3. Experiment 1: alkaloid analyses

Nicotine and minor alkaloid levels in Kodiak and Camel Snus extract stock solutions were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) by modification of a previously described method (Rangiah et al., 2011). Briefly, the extracts were mixed with stable isotope-labeled nicotine and nornicotine, anatabine, and anabasine (internal standards), diluted with 10 mM ammonium acetate containing 5% methanol, and analyzed by LC-MS/MS on a Hypercarb column (Thermo Scientific), using 10 mM ammonium acetate (with 0.001% formic acid) and methanol as mobile phase.

2.4. Experiment 2: effects of nicotine alone and Kodiak extract on ICSS

2.4.1. Intracranial self-stimulation. Surgery, apparatus, and training procedure used here are described in detail elsewhere (Harris et al., 2010, 2011; Roiko et al., 2009). Briefly, animals were anesthetized with i.m. ketamine (75 mg/kg)/xylazine (7.5 mg/kg) and implanted with a bipolar stainless steel electrode in the medial forebrain bundle at the level of the lateral hypothalamus. Rats were later trained to respond for electrical brain stimulation using a modified version of the Kornetsky and Esposito (1979) discrete-trial current-threshold procedure (Markou and Koob, 1992). Each session was approximately 45 min and provided two dependent variables: ICSS thresholds (a measure of brain reinforcement function) and response latencies (a measure of non-specific, e.g. motor effects).

2.4.2. Experiment 2a: First assessment. Animals ($N = 12$) were tested in daily ICSS sessions conducted Mon-Fri until thresholds were stable (i.e. less than 10% coefficient of variation over a 5-day period and no apparent trend). To habituate animals